Metformin ameliorates olanzapine-induced insulin resistance via suppressing macrophage infiltration and inflammatory responses in rats

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ARTICLEINFO

Keywords:
Olanzapine
Metformin
Insulin resistance
Macrophage infiltration
Rats

ABSTRACT

Aims: The present study aimed to investigate the possible effects of metformin on the olanzapine-induced insulin resistance in rats.

Methods: Rats were randomly divided into three groups: the control (Control) group, the olanzapine (Ola) group and the olanzapine + metformin (Ola + Met) group. Rats in the Ola group received olanzapine (8 mg/kg/day) intraperitoneally while rats in the Ola + Met group received olanzapine (8 mg/kg/day) intraperitoneally and metformin (300 mg/kg/day) orally for 8 weeks. Rats in the Control group received vehicle accordingly. Body weight and fasting blood glucose were recorded routinely. Inflammatory cytokines TNF-α, IL-6 and IL-1β and IL- 10 were measured by ELISA. The gene expression of macrophages markers was examined by qPCR. The epididymal white adipose tissue, liver and skeletal muscle were also isolated for immunohistochemical analysis. *Results:* Olanzapine significantly induced body weight gain and insulin resistance compared to the control, which was markedly alleviated by metformin. Pro-inflammatory cytokines TNF-α, IL-6 and IL-1β were upregulated while the anti-inflammatory cytokine IL-10 was downregulated by olanzapine in plasma and epididymal white adipose tissue compared to the control, but not the liver and skeletal muscle. However, metformin co- administration significantly decreased the levels of TNF-α, IL-6 and IL-1β while increased the level of IL-10 in epididymal white adipose tissue compared to olanzapine-treated rats. Moreover, olanzapine treatment markedly increased the expression of the CD68 and the M1 macrophage markers while decreased the expression of the M2 macrophage markers in epididymal white adipose tissue in rats compared to the control. However, metformin co- treatment ameliorated the effects of olanzapine.

Conclusions: Our results suggest that metformin alleviated olanzapine-induced insulin resistance possibly by suppressing the inflammatory responses mediated by macrophage infiltration and polarization in epididymal white adipose tissue.

1. Introduction

Atypical antipsychotics are frequently associated with metabolic disorders, such as weight gain, glucose intolerance, insulin resistance, and metabolic syndrome. The prevalence of metabolic disorders is ~35.3 % among people taking antipsychotics, which is more than 3-fold higher than the unmedicated schizophrenia patients [1,2]. Among atypical antipsychotics, olanzapine is widely ascribed for schizophrenia and other psychiatric disorders and produces the most serious abnormalities in glucose and lipid metabolism [3,4]. Olanzapine-induced metabolic disorders and insulin resistance are frequently associated with a chronic low-grade inflammation, which is likely initiated in the adipose tissue [5,6]. Indeed, numerous studies have demonstrated that olanzapine treatment induced extensive macrophage infiltration and a significant increase of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) expression in adipose tissue in rats [5, 7].

Macrophages are now emerging as an important player in the pathogenesis of inflammation and insulin resistance [8]. Accumulation of macrophages in adipose tissue is associated with body weight gain and causes insulin resistance in both human and rodents [9]. Among the tissue macrophages there are two main subtypes that are capable of dynamic interconversion: the classically activated macrophages (M1) and the alternatively activated macrophages (M2) [10]. M1 macrophages secrete proinflammatory cytokines, including TNF-α, IL-1β, and IL-6. These cytokines activate inflammatory signaling pathways such as JNK pathways to impair insulin action and cause insulin resistance [11,12]. M2 macrophages produce antiinflammatory cytokines, such as IL-10 and arginase-1 (Arg-1) [13]. Maintenance of the balance between M1 and M2 macrophages is essential for glucose homeostasis because the dominance of the M1 phenotype leads to the development of insulin resistance [14,15].

Metformin has been widely used for patients with type 2 diabetes mellitus. In addition to glycemic control, metformin has also been shown to have antiinflammatory effects in clinical studies and preclinical studies [16–19]. It seems that metformin ameliorated inflammation-associated disorders such as obesity and atherosclerosis at least in part by modulating macrophage infiltration and polarization [20,21]. Previous studies have reported that metformin significantly attenuated olanzapine-induced body weight gain and insulin resistance in human as well as in rodents [22,23]. However, the underlying mechanisms remain unclear.

The present study was undertaken to evaluate the effect of metformin on insulin resistance induced by olanzapine treatment and explore the underlying mechanisms by examining the inflammatory responses, macrophage infiltration and polarization.

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https://doi.org/10.1016/j.biopha.2020.110912
Received 12 July 2020; Received in revised form 13 October 2020; Accepted 17 October 2020
Available online 17 November 2020

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2. Materials and methods

2.1. Reagents

Olanzapine (O126519) and metformin (M107827) were purchased from Aladdin (Shanghai, China). Chloroform (10,006,818), isopropanol (80,109,218), and anhydrous ethanol (10,009,218) were obtained from Sinopharm (Wuhan, China). All drugs and chemicals used were of analytical grade. Rat ELISA kits were purchased from ELK Biotechnology: Insulin (ELK2370); TNF-α (ELK1396); IL-6 (ELK1158); IL-1β (ELK1272); IL-10 (ELK1144). The sensitivity of ELISA kits for Insulin, TNF-α, IL-6, IL-1β, and IL-10 were 49.3 pg/mL, 6.1 pg/mL, 3.3 pg/mL, 5.9 pg/mL, and 3.1 pg/mL respectively. The ELISA kits had high sensitivity and excellent specificity for detection. No significant cross-reactivity or interference was observed. TRIpure Total RNA Extraction Reagent (EP013) was purchased from ELK Biotechnology. Antibody against CD68 (ab125212, 1:150 dilution) was purchased from Abcam (Cambridge, USA). Peroxidase-conjugated secondary antibodies (AS-1107, 1:200 dilution) were obtained from Aspen Technology (Wuhan, China).

2.2. Animals

Female SD rats, 8 weeks old, weighing approximately 200 g were purchased from Beijing Huafukang Biotechnology Co., Ltd. and housed individually under a 12 h light/dark cycle (lights on at 8:00 h) at 22 ± 2℃. Food and water were accessed *ad libitum*. Animals were routinely acclimated to laboratory conditions for 1 week before the experiments. Rats were randomly assigned to three groups (8 per group) as follows: the control group (Control group), the olanzapine group (Ola group), and the olanzapine + metformin group (Ola + Met group). All animal experimental procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The animal facilities and protocol were approved and guided by the Institutional Animal Care and Use Committee of the Tongji Medical College, Huazhong University of Science and Technology (No. XH-B20150422).

2.3. Drug treatment

Olanzapine was dissolved in 0.1 N HCl in distilled water, adjusting to pH 6.0 with 1 N NaOH. The vehicle was similarly pH-adjusted, distilled water. The dose of olanzapine (8 mg/kg/day) was chosen to represent the physiologically relevant level in vivo, as well as our previous studies [24,25]. Olanzapine was administrated intraperitoneally in a volume of 1 mL/kg. Metformin was dissolved in 0.9 % saline solution and administered orally (gastric gavage) in a volume of 1 mL/kg for 8 weeks. The dose of metformin (300 mg/kg/day) was based on evidence that similar doses significantly prevented olanzapine-induced insulin resistance [26,27]. The vehicle for metformin was 0.9 % saline. Eight rats were gavaged with vehicle, and injected intraperitoneally with vehicle to serve as controls.

2.4. Metabolic measures

Body weight and fasting blood glucose were routinely recorded. After the rats were fasted for about 14 h, blood samples were collected from tail tip incision at set time points. Briefly, the rat was fixed in a v- trap and the tip of the tail was amputated. Then the blood was drawn from the pool of blood on the tip of the tail. If necessary, the blood was moved to the tip of the tail by gently “milking” the tail. Subsequent samples were drawn by removing the clot or re-cutting when necessary. Then the blood glucose was assessed by using the glucometer (One Touch Ultra, USA). For oral glucose tolerance test (OGTT), rats were fasted for 14 h and then gavaged with glucose (2 g/kg). Blood samples were collected via a tail snip at 0, 30, 60, 90, and 120 min and glucose levels were determined. Individual glucose measurements at the 5 time points during the OGTT were integrated to generate a single area under the curve (AUC) value. The AUC value was calculated according to trapezoidal rules: the total area under the curve was the sum of all individually calculated trapezoid areas; the individual trapezoid area equals (((y value two + y value one)/2)*(x value two – x value one)) [28]. The homeostatic model assessment of insulin resistance (HOMA IR) index was calculated: [fasting insulin (mIU/L) × fasting glucose (mmol/L)]/22.5 [29]. A larger calculated HOMA IR index value denotes greater insulin resistance.

2.5. Tissue collection

Rats were anesthetized by 1.5 %–2.5 % (vol/vol) isoflurane mixed with air at the end of the experiments [30]. Blood samples were collected from the abdominal aorta by disposable polypropylene syringes and plasma was isolated by centrifugation at 1800×g for 10 min at 4℃. The plasma was store at -80℃ for further analysis. After the blood was collected, the rats were sacrificed. The liver, skeletal muscle and epididymal white adipose tissues (eWAT) were dissected and separated to two halves: one half was snap frozen in liquid nitrogen and stored at -80℃ until further analysis, and the other half was fixed overnight by immersion at 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Then the fixed samples were dehydrated, cleared and embedded in paraffin for further assessment. *2.6. Enzyme-linked immunosorbent assay (ELISA)*

The levels of insulin in plasma and inflammatory cytokines (TNF-α, IL-6, IL-1β and IL-10) in plasma, liver, skeletal muscle, and eWAT were estimated by the respective rat ELISA kits according to the manufacturer’s instructions. Briefly, samples and cytokine standards were added to the 96-well plates coated with monoclonal antibody and incubated at 37℃ for 30 min. Then the plates were washed and incubated with the substrate solution at 37℃ for 20 min. Finally, the stop solution was added and the absorbance was determined at 450 nm by a microplate reader (DR-200Bs, Diatek, China). Results were plotted against the linear portion of the standard curve. Each sample was measured in triplicate.

2.7. Immunohistochemistry

The paraffin-embedded tissues (liver, skeletal muscle and eWAT) were section-sliced (4 um/section) at 40 um intervals. Following blocking with 0.3 % H_2O_2/methanol for 30 min at room temperature, specimens were blocked with PBS containing 5% normal horse serum for 30 min at room temperature. Anti-CD68 was added to the specimen sections at 4℃ and incubated overnight. Then specimens were briefly washed in PBS and incubated with polymer detection system (1:1 dilution) for 30 min at room temperature. The specimens were washed with PBS and developed using diaminobenzidine solution for 2 min at room temperature. Finally, specimens were subsequently washed with water and counterstained with hematoxylin for 30 s at room temperature.

Table 1
Sequences of primers for RT-PCR.

primers		sequences
CD68	forward	5′-CAGTGGACATTCTCAGCGCA-3′
	reverse	5′-GTAACGCAGAAGGCAATGAGC-3′
CD11c	forward	5′-TGAGGGAACTCAATCAAGGTCA-3′
	reverse	5′-AGGTGGGTCTCGTATTTGGG-3′
CCR7	forward	5′-TGGTGGCTCTCCTGGTCATT-3′
	reverse	5′-TGAGTACATGAGAGGGAGGAACC-3′
CD206	forward	5′-GCCTACTGCCTGCCCTAATC-3′
	reverse	5′-CCCATCGCTCCACTCAAAGT-3′
Arg1	forward	5′-ATTGGCAAAGTGATGGAAGAGC-3′
	reverse	5′-CAAGACAAGGTCAACGCCAC-3′
GAPDH	forward	5′-GCCAAGGTCATCCATGACAAC-3′
	reverse	5′-GTGGATGCAGGGATGATGTTC-3′

2.8. RNA extraction and Gene expression analysis

Tissue samples (generally 20 mg) were suspended in 1 mL Trizol solution and homogenized with a tissue homogenizer until no solid tissue was observed. The mRNA was extracted by TRIpure Total RNA Extraction Reagent following the protocol recommended by the manufacturers. Then RNA was reverse-transcribed into cDNA using the EntiLink 1st Strand cDNA Synthesis Kit (ELK Biotech, China) according to manufacturer's protocol. qRT-PCR was performed using SYBR Green PCR SuperMix (ELK Biotech, China) and a StepOne Real-Time PCR System (Life technologies, USA). The results were calculated using the $2^{-\Delta\Delta CT}$ method. GAPDH was used as the internal control to normalize the expression data. The primer sequences are given in Table 1.

2.9. Statistical analysis

Statistical analysis was performed by SPSS 19.0 software (IBM, IL, USA). Data were presented as mean ± SEM. The normality of distribution of data was checked by a one-sample Kolmogorov-Smirnov test. Differences among multiple groups were determined by one-way ANOVA followed by Tukey's multiple-comparison *post hoc* test. Correlations were analysed using Pearson's correlation. P 0.05 was considered statistically significant.

3. Results

3.1. Metformin alleviates olanzapine-induced body weight gain

In our study there was not any observed mortality during the course of the experiment. Olanzapine treatment for eight weeks significantly increased body weight compared to the control. However, metformin treatment led to a significant reduction in body weight as compared to olanzapine-treated group (Fig. 1A, 1B). Furthermore, olanzapine caused a significant increase of percentage body weight gain which was significantly reduced by metformin treatment compared to olanzapine-

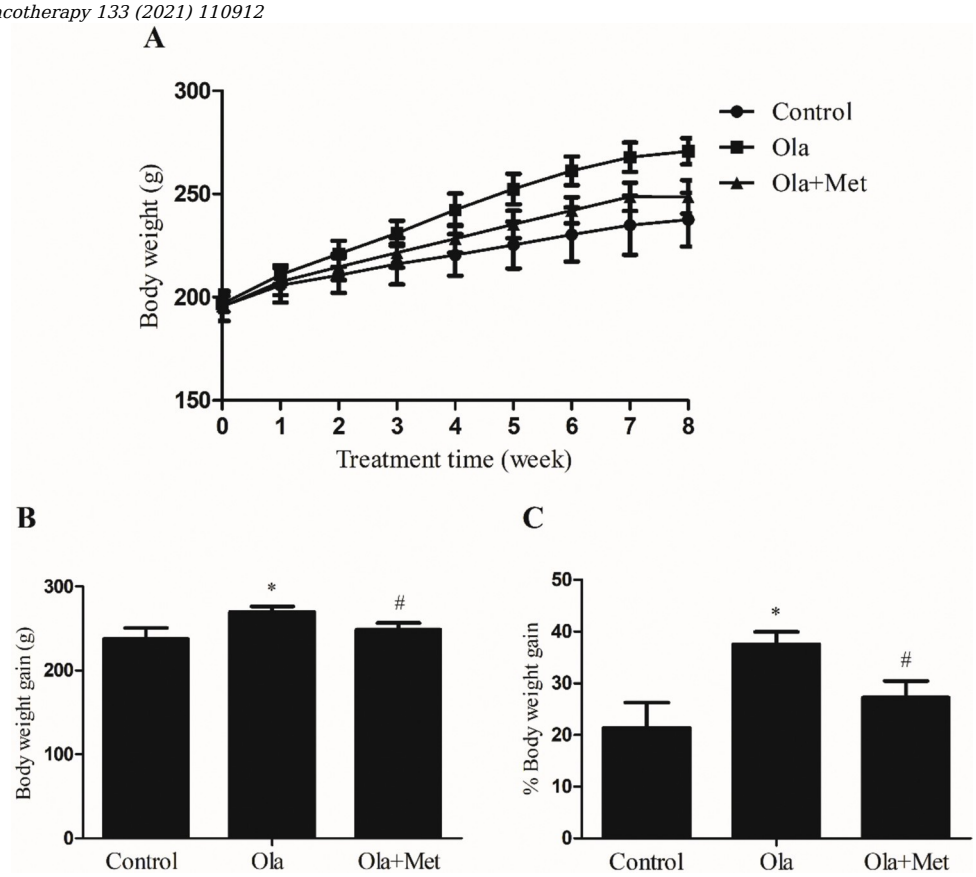


Fig. 1. Effect of olanzapine and metformin treatment on body weight and percentage weight gain. n = 8. *p < 0.05 vs. the control group; #p < 0.05 vs. the olanzapine-treated group.

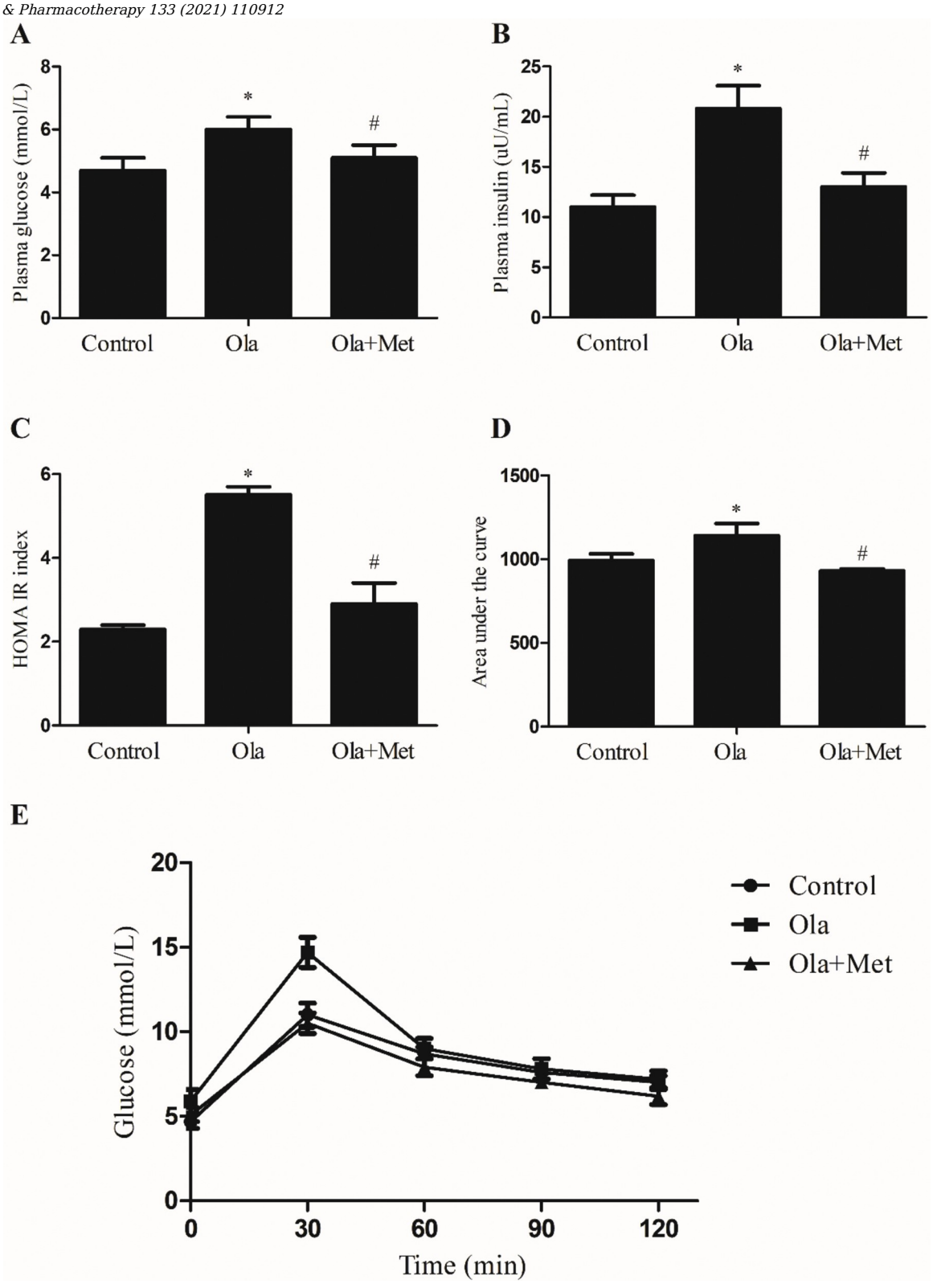
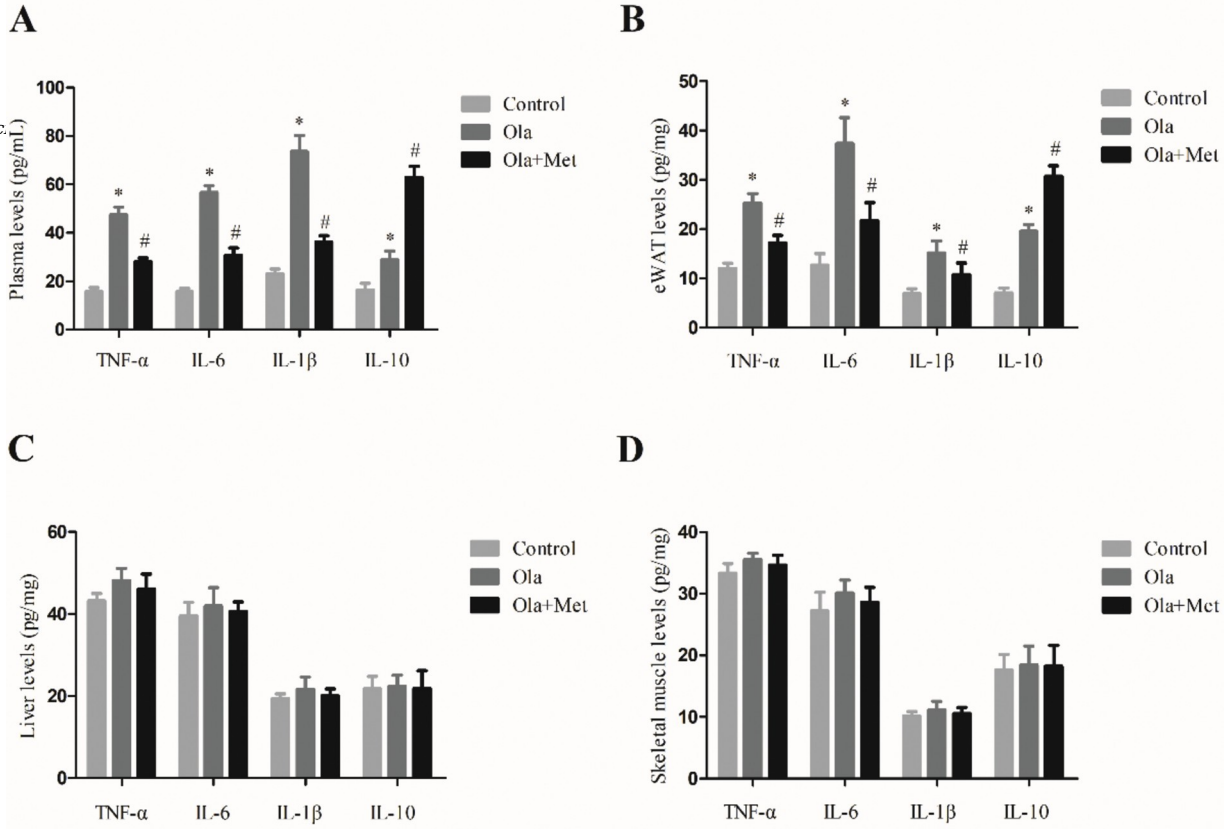


Fig. 2. Effect of olanzapine and metformin treatment on fasting blood glucose, insulin, HOMA IR index, OGTT and AUC. n = 8. *p < 0.05 vs. the control group; #p < 0.05 vs. the olanzapine-treated group.

treated group (Fig. 1C).

3.2. Metformin alleviates olanzapine-induced glucose intolerance and insulin resistance

Compared with the control group, rats displayed higher levels of fasting blood



glucose and insulin, impaired glucose tolerance and insulin

Fig. 3. Effect of olanzapine and metformin treatment on levels of inflammatory cytokines in plasma, eWAT, liver and skeletal muscle. ELISA was used to detect the levels of pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and anti-inflammatory cytokine IL-10 in plasma, eWAT, liver and skeletal muscle of rats. n = 8.
*p < 0.05 vs. the control group; #p < 0.05 vs. the olanzapine-treated group.

sensitivity after olanzapine treatment. Compared with olanzapine treatment, metformin treatment significantly lowered blood glucose and insulin levels, improved glucose tolerance and insulin sensitivity (Fig. 2A, B, D, E). Furthermore, we observed that the HOMA IR index was significantly increased in the olanzapine-treated group, whereas metformin treatment significantly reversed the increase of HOMA IR index (Fig. 2C).

3.3. Metformin reduces olanzapine-induced inflammation in plasma and eWAT

Olanzapine treatment significantly increased plasma levels of TNF-α, IL-6 and IL-1β with a concomitant decreased IL-10 level compared to the control group. Metformin co-treatment significantly reduced the elevated plasma levels of TNF-α, IL-6 and IL-1β while reversed the decreased IL-10 level compared to the olanzapine-treatment group (Fig. 3A). Similarly, in the eWAT, the levels of TNF-α, IL-6 and IL-1β were significantly elevated and IL-10 level was significantly reduced by olanzapine treatment compared with the control group. Compared with the olanzapine-treated group, the levels of TNF-α, IL-6 and IL-1β were significantly downregulated while the IL-10 level was significantly upregulated in the eWAT by metformin (Fig. 3B). However, in the liver and skeletal muscle, there was no significant difference in terms of the levels of TNF-α, IL-6, IL-1β and IL-10 among the groups (Fig. 3C, D).

3.4. Metformin reduces olanzapine-induced macrophage infiltration and M1 polarization in the eWAT

To explore the possible mechanism for the antiinflammatory effect of metformin, we examined the effects of metformin on macrophage infiltration and polarization in eWAT. Compared with the control group, olanzapine significantly increased the number of CD68+ cells in the eWAT. Metformin administration led to a significant decrease in the number of CD68+ cells compared to the olanzapine-treated group (Fig. 4A, B). PCR also revealed a significant increased accumulation of macrophages in eWAT in olanzapine-treated rats compared with the control rats with increased mRNA expression of CD68, which was significantly reversed by metformin administration (Fig. 4D). Additionally, the accumulation of macrophages in the skeletal muscle was relatively unchanged (Fig. 4C). However, we did not observe accumulation of macrophages in the liver after olanzapine and metformin treatment (Fig. 4A). Moreover, gene expression results demonstrated that metformin decreased the expression of genes associated with M1 polarization (CD11c, CCR7) and increased those associated with M2 polarization (CD206, Arg1) in eWAT in olanzapine-treated mice (Fig. 4E). These results confirm that metformin attenuated macrophage infiltration and M1 polarization in eWAT in olanzapine-treated rats. Furthermore, we carried out correlation analysis on inflammatory cytokines and gene expression in eWAT (Table 2). The results showed that a significant correlation was found between CD68 expression and inflammatory cytokines (TNF-α, IL-6, IL-1β and IL-10). A significant correlation was also found for inflammatory cytokines (TNF-α, IL-6 and IL- 1β) and gene expression (CD11c, CCR7, CD206 and Arg1). Moreover, a significant correlation was also found

for IL-10 and CD206 and Arg1. However, no significant correlation was observed between IL-10 and CD11c, CCR7.

4. Discussion

In the present study, we investigated the effects of metformin on insulin resistance induced by olanzapine treatment. Our results suggested that metformin significantly attenuated olanzapine-induced

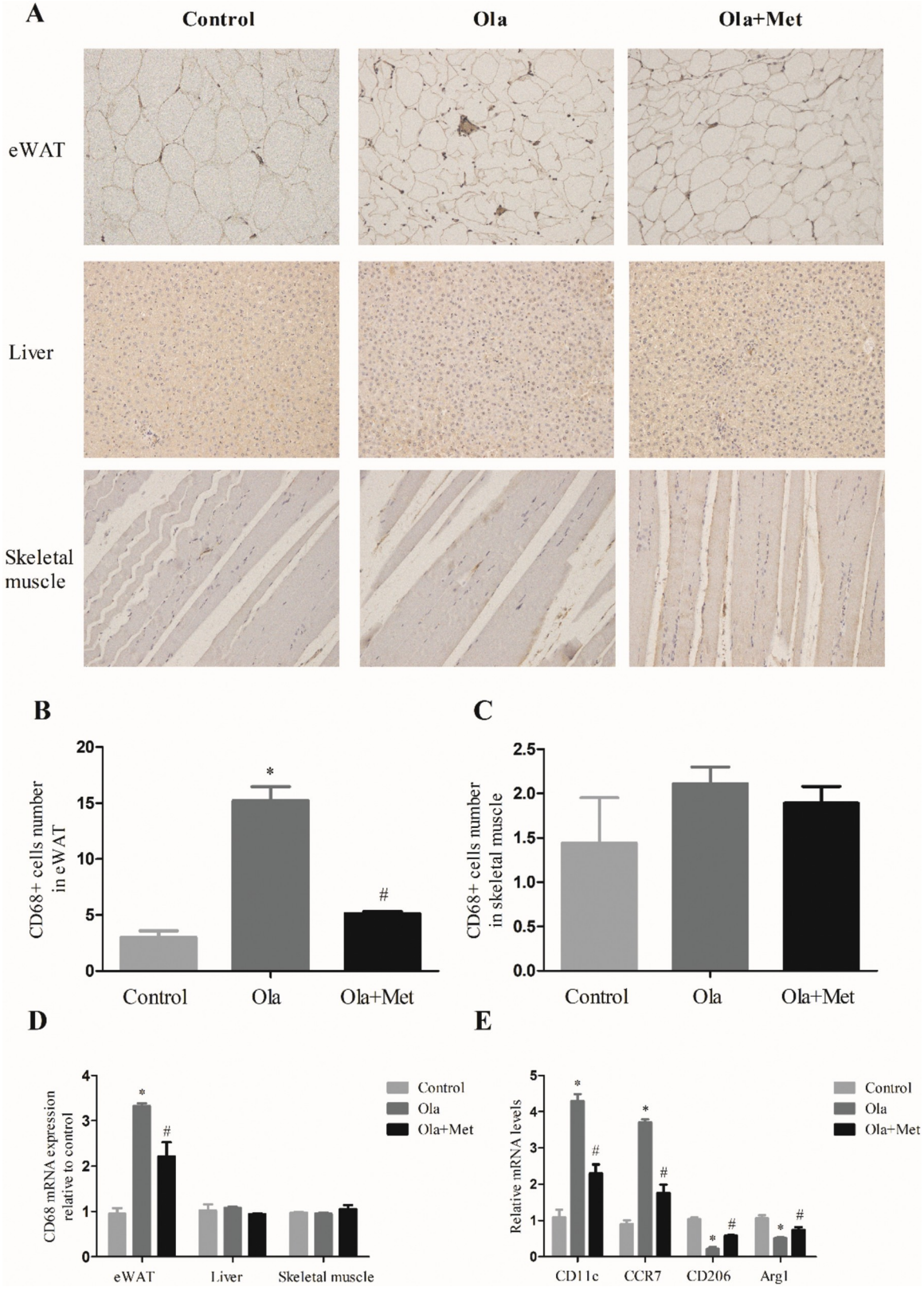


Fig. 4. Effect of olanzapine and metformin treatment on macrophage infiltration and polarization in rats. (A) CD68 immunohistochemistry was performed to investigate the effect of olanzapine and metformin on macrophage infiltration in eWAT, liver and skeletal muscle. (B) Quantification of CD68+ cells in eWAT. (C) Quantification of CD68+ cells in skeletal muscle. (D) The CD68 mRNA expression in eWAT, liver and skeletal muscle was detected by PCR. (E) Effect of olanzapine and metformin on gene expression associated with macrophage polarization in eWAT. qPCR was used to detect the mRNA expression of CD11c, CCR7, CD206, Arg1 in the eWAT of rats. n = 8. *p < 0.05 vs. the control group; #p < 0.05 vs. the olanzapine-treated group.

Table 2
The correlation analysis between related genes expression (CD68, CD11c, CCR7, CD206, Arg1) and changes of inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-10) in the eWAT.

	Correlation coefficient							
	TNF-α	IL-6	IL-1β	IL-10				
CD68	0.943*	0.924*	0.875*	0.544*				
CD11c	0.969*	0.941*	0.855*	0.395	CCR7	0.972*	0.947*	0.861*
0.332								
CD206	− 0.944*	− 0.909*	− 0.869*	− 0.611*	Arg1			
	− 0.933*	− 0.899*	− 0.828*	− 0.575*				

* p□0.05.

insulin resistance via suppressing the low-grade inflammation and the underlying mechanisms might be associated with the alleviation of macrophage infiltration and M1 polarization in white adipose tissue.

Weight gain induced by olanzapine treatment may cause medication non-adherence and increase the risk of insulin resistance, diabetes and cardiovascular disease. Previous studies have shown that metformin is effective and safe in improving weight gain and insulin resistance induced by olanzapine in the schizophrenic population [31,32]. The exact molecular mechanisms responsible for its improvement of olanzapine-induced insulin resistance are not completely understood but may include the alleviation of weight gain, although it has been indicated that chronic olanzapine treatment could induce insulin resistance independent of weight gain [33,34]. However, in some clinical trials metformin did not attenuate olanzapine-induced weight gain and exhibited no significant benefit compare with placebo [35,36]. Further studies are needed to estimate the effect of metformin on olanzapine-induced weight gain and insulin resistance.

The low-grade inflammation and the increased proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 have been repeatedly associated with an increased risk of insulin resistance [37–39]. Previous studies have reported that olanzapine treatment significantly upregulated the levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in serum, hypothalamus, WAT, and BAT [7]. Our previous studies also shown that olanzapine significantly increased circulating levels of TNF-α, IL-1β, and IL-6 in humans as well as in rodents [40]. Consistently, in the present study we found that the levels of TNF-α, IL-1β, and IL-6 in plasma and eWAT were significantly upregulated by olanzapine treatment compared to the control rats. Moreover, compared to the control rats the antiinflammatory cytokine IL-10 in plasma and eWAT was significantly reduced by olanzapine treatment. However, metformin co-administration significantly decreased the levels of TNF-α, IL-1β, and IL-6 in plasma and eWAT, whereas increased the levels of IL-10 in plasma and eWAT in olanzapine-treated rats. Although the crucial mechanisms underlying the inhibition by metformin of olanzapine-induced insulin resistance remain to be elucidated, our study may imply that such antiinflammatory properties could contribute, at least in part, to the inhibition of metformin beyond glucose lowering. Interestingly, there was no significant difference in terms of the levels of TNF-α, IL-1β, IL-6, and IL-10 in the liver and skeletal muscle between the olanzapine-treated rats and the control rats. Similarly, Qingsheng Zhang et al. reported that olanzapine treatment increased the mRNA expression of TNF-α, IL-1β, IL-6 in WAT, but not in the liver [7]. These results suggest that olanzapine-induced insulin resistance is not associated with liver or skeletal muscle inflammation. Future studies are warranted to investigate the underlying mechanism for this difference.

In inflammatory adipose tissue, non-fat cells such as macrophages account for nearly all pro-inflammatory cytokine production [41]. Macrophage infiltration and polarization to the pro-inflammatory M1 phenotype instead of the anti-inflammatory M2 phenotype in adipose tissue have been closely associated with chronic low-grade inflammation and metabolic disorders [9,41]. Previous studies have reported that olanzapine treatment is associated with increased CD68+ cells and CD68 mRNA expression in WAT in rats [5,6]. These findings are consistent with the results in our study. Moreover, olanzapine treatment resulted in an increased expression of genes associated with M1 polarization, whereas expression of genes associated with M2 polarization were decreased in olanzapine-treated mice compared with the control mice. However, metformin co-administration decreased the expression of CD68 and M1 marker genes while increased the expression of M2 marker genes in eWAT in olanzapine-treated rats. These data demonstrate that olanzapine promotes macrophage infiltration and M1 polarization in eWAT in rat, which may contribute to the inflammation associated with insulin resistance. In addition, metformin decreased the macrophage infiltration and M1 polarization while increased M2 polarization in eWAT in olanzapine-treated rats. Moreover, significant correlation was found between the expression of CD68 and M1/M2 marker genes and inflammatory cytokines in eWAT, indicating that the decreased CD68 expression and altered M1/M2 phenotype switch were significantly associated with a marked alleviation of inflammation in eWAT. Thus, consistent with previous studies [20,42], our results suggest that metformin displays anti-inflammatory potentials at least in part by modulating macrophage infiltration and switching the polarization from M1 phenotype to M2 phenotype. However, we did not observe significant difference in macrophage infiltration in the skeletal muscle between the olanzapine-treated rats and the control rats, Moreover, our results indicated that macrophage infiltration was absent in the liver after olanzapine treatment, which is

consistent with a previous report that olanzapine treatment increased macrophage infiltration in WAT but not liver [7]. Our study, together with previous reports, may suggest that the liver and skeletal muscle does not contribute significantly to the insulin resistance and metabolic disorders induced by olanzapine treatment.

Our results indicated that olanzapine regulates at least two stages of adipose tissue inflammation: 1) macrophage infiltration and 2) macrophage subtype transformation, but how olanzapine exerts these effects is unclear. It has been reported that activation of the NF-κB-IL6-STAT3 axis was involved in M1 polarization [43]. NF-κB has key roles in regulating innate immune and inflammatory responses. Meanwhile, NF-κB is an important transcription factor that up-regulated the expression of proinflammatory cytokines such as TNF-α and IL-6 [44]. Previous studies have shown that olanzapine treatment significantly upregulated NF-κB expression in serum and hypothalamus in rats [45]. Our previous study also showed that olanzapine treatment significantly stimulated NF-κB activity in WAT in rats [40]. Considering that metformin could inhibit NF-κB expression and activation [46,47], NF-κB may also be responsible for the inhibition of metformin of olanzapine-induced inflammation and insulin resistance. Future studies are warranted to investigate the mechanism of olanzapine-induced macrophage infiltration and M1 polarization.

There are some limitations that need to be addressed. First, our study could not clearly identify whether weight loss plays a part in the alleviation of olanzapine-induced insulin resistance by metformin. More study is needed to show that metformin improves olanzapine-induced insulin resistance independently of weight loss. However, in our study, our results suggested that inflammation at least in part was involved in the alleviation of olanzapine-induced insulin resistance by metformin. Second, we did not measure the adipocyte size which was closely associated with macrophage infiltration in eWAT. However, in our study we investigated the mRNA expression of CD68 and CD68+ cells in the eWAT which may partially address the concern. Finally, the proportion of M1 and M2 macrophages in the eWAT were not detected by flow cytometry. However, the expression profile of macrophage markers partially indicates the effect of olanzapine and metformin on macrophage polarization in eWAT.

In conclusion, the present study demonstrated that metformin markedly alleviated the body weight gain and inflammation in olanzapine-treated rats, which may contribute to the improved insulin resistance and metabolic disorders. The anti-inflammatory benefits of metformin may be related to an alteration in macrophage infiltration and M1-M2 phenotype switch in epididymal white adipose tissue. The present study confirms the low grade inflammatory response and extends our knowledge of inflammation under olanzapine treatment. Future investigations are needed to elucidate the involved signaling pathways between olanzapine and macrophage infiltration and polarization.

Declaration of Competing Interest

All authors declare no conflicts of interest in this study.

Acknowledgements

This work was supported by the grants from the National Natural Science Foundation for Young Scientists of China (81803637).

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